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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

POLO et al.

For: COMPOSITIONS AND METHODS FOR GENERATING AN IMMUNE RESPONSE UTILIZING ALPHAVIRUS-BASED VECTOR SYSTEMS

Serial No.: 09/551,977

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Examiner: B. Li

Group Art Unit: 1648

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DECLARATION OF JOHN POLO PURSUANT TO 37 C.F.R. § 1.132

Mail Stop Non-Fee Amendment
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Alexandria, VA 22313

DECLARATION PURSUANT TO 37 C.F.R. § 1.132 OF JOHN M. POLO, Ph.D.

Dear Sir:

I, John M. Polo, hereby declare as follows:

1. I received my Ph.D. in Virology from North Carolina State University in 1990. I am currently Director, Vaccine Research at Chiron Corporation in Emeryville, CA and have been at Chiron since 1995. Before joining Chiron, I was a Scientist at Viagene, Inc., in San Diego, CA. A copy of my Curriculum Vitae (Exhibit A) is attached hereto.

2. I am extremely familiar with studies of virology, virus vectors and vaccines, having worked in these disciplines for almost 20 years. I have also co-authored numerous publications and patents in these fields.

3. I have reviewed the pending Patent Application Serial No. 09/551,977 for "COMPOSITIONS AND METHODS FOR GENERATING AN IMMUNE RESPONSE UTILIZING ALPHAVIRUS-BASED VECTOR SYSTEMS" (herein after the "specification") and the currently pending claims. I have also reviewed the Office Action dated January 27, 2003

and the references cited therein including Tucker *et al.*, *J. Virol.* 1997, Vol. 71, pp. 6106-6112 (hereinafter "Tucker") and MacDonald *et al.*, *J. Virol.* 2000, Vol. 74, pp. 914-922 (hereinafter "MacDonald"). Therefore, I am familiar with the issues raised by the Examiner in the Office Action.

4. I understand that the pending claims are directed to recombinant alphavirus particles that are capable of infecting human dendritic cells. In addition, the claimed particles include an amino acid mutation the E2 glycoprotein as compared to the wild-type alphavirus sequence from which the recombinant particles are derived. Specifically, the mutation(s) are found in one or more residues that correspond to amino acids residues numbered 158 to 162 in Sindbis (SIN). The pending claims exclude recombinant alphavirus particles that are derived from ATCC # VR-2526.

5. It is my opinion that, as a technical matter, a skilled worker could have readily made and used the compositions of the pending claims in light of the specification, together with the common general knowledge, tools and methods available as of the effective filing date of April 1999. It is further my opinion that the references cited by the Examiner (Tucker and MacDonald) do not teach or suggest problems with the enablement of the pending claims. I base these opinions on the facts set forth below; however, I call attention to the fact that it was considered routine experimentation at the time of filing to culture human dendritic cells and to introduce specific mutations in amino acid sequences. I also call attention to the fact that the specification provides abundant direction regarding testing the ability of an alphavirus particle to infect human dendritic cells. In drawing my conclusions, I have considered the nature of the claims, the quantity of experimentation required to practice the subject matter of the claims, the direction present in the specification, the state of the field at the time the application was filed, the teachings of the cited references and the level of skill in the art.

6. At the outset, I note that the term "skilled worker" is a worker with a routine level of skill in the fields of molecular biology and virology in April 1999 with a Ph.D. degree and two or more years of post-doctoral training. In view of my training and experience, I am currently, and was in April 1999, such a skilled worker.

7. When the specification was filed, it clearly taught a typical scientist how to make and use recombinant alphavirus particles from a variety of alphavirus species, where the particles are capable of infecting human dendritic cells and contain an amino acid mutation at positions 158-162 (based on SIN numbering) of E2 (relative to the wild-type alphavirus source). Thus, I believe that a typical scientist would have understood the specification clearly described all of the various aspects of the claims and enabled a typical scientist to make and use the invention as set forth in the pending claims. I base this belief on the facts set forth below.

8. First, at the time the specification was filed, both the nucleotide and the amino acid sequence of the E2 proteins of a many alphaviruses were known and published. (See, for example, page 21 and background section of the specification). The amino acid sequences of any alphavirus that was not known could have been easily obtained by standard sequencing techniques using RNA isolated from alphaviruses. It was also known at the time of filing that Sindbis (SIN) was considered the prototype and model for other alphaviruses. (See, *e.g.*, page 2, lines 8-16 of the specification). In view of the teachings of the specification, it would have been routine for the skilled artisan to align and compare nucleotide and amino acid sequences from various alphaviruses and determine which amino acid sequences in any alphavirus corresponded to positions 158-162 of a SIN E2 protein. (See, *e.g.*, page 37 of the specification, describing alignment of SIN strains). Also, in view of the disclosure, a person of skill in the art would surmise that mutants in this region would be much more likely to exhibit DC-tropism. Accordingly, it is my opinion that using the teachings of the specification and state of the art, it would require only routine experimentation for a typical scientist to obtain suitable amino acid sequences from any alphavirus (for example by comparison with sequences disclosed in the specification) and use these alphavirus sequences as a starting point for making the claimed particles.

9. Second, in light of the teachings of the specification, it would have been routine for a typical scientist to mutate one or more of amino acid residues corresponding to residues 158-162 of E2. Methods of making amino acid mutations were well known in the art and described, for instance in Example 1 (particularly page 34) of the specification. Further, the production and testing of a particular mutant at residue 160 is described in the specification. It would also have been routine to determine, by structural and/or functional analyses, which amino

acids corresponding to 158-162 could be mutated to develop DC-tropic particles. In light of the teachings of the specification, I believe that a typical scientist would have known how to make and use alphavirus particles including mutations in the specified region of an alphavirus E2.

10. Third, it would have been routine to produce alphavirus particles having the claimed mutation(s) in residues 158-162 of E2. Methods of generating (packaging) recombinant alphavirus particles, for example, through co-transfection of complementing vector and defective helper (DH) molecules or by introduction of vector into stable packing cell lines, were well known at the time of filing. (See, also, page 23, lines 5 to 23 of the specification). Also well known were methods of performing site-directed mutagenesis that would target one of the residues at positions 158-162. Thus, it is my opinion that it would have been routine for the skilled artisan to make recombinant alphavirus particles having the claimed mutation(s) in positions 158-162 of E2.

11. Fourth, it would have been clear to a typical scientist how to test for the ability of a mutant alphavirus particle falling within the scope of the claims to infect human dendritic cells. (See, *e.g.*, page 42 of the specification). Methods of culturing human dendritic cells were known and described in the specification as filed. (See, *e.g.*, Example 1). Moreover, methods of testing the ability of alphavirus particle to infect these cells are described in detail in the specification and include, but are not limited to, testing FACS analysis, titer analysis, use of reporter molecules, and the like. (See, *e.g.*, page 40; page 42-43). Thus, it is my opinion that a typical scientist could have readily tested any recombinant alphavirus particle containing the claimed mutation, following the teachings of the specification.

12. It is further my opinion that MacDonald and Tucker are not relevant to the claimed recombinant alphavirus particles. Neither reference discloses infection of human dendritic cells with recombinant alphavirus particles, as required by all the pending claims. Furthermore, neither references describes, demonstrates or suggests what effect mutations in the claimed region of 158-162 would have on DC-tropism. In this regard, MacDonald discloses mutations only at positions 76 and 116 of E2, while Tucker discloses mutations at positions 55 or 172 of E2.

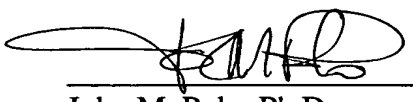
13. In addition, it is my opinion that it would have been clear to the skilled worker at the time the specification was filed that the claims encompassed mutations at one or more of the residues corresponding to residues 158-162 of an alphavirus E2 glycoprotein. It is clear from the specification, for example, on page 5, lines 4-12 that "an" amino acid substitution refers to one or more substitutions in the specified region.

14. Thus, it is my opinion that making and using recombinant alphaviruses of the claimed invention was a predictable art. I have no doubt that as of April, 1999 a person of skill in the art was capable of making the alphavirus mutants and testing them for the ability to infect human dendritic cells. Even if a mutant were inoperable for some reason, e.g., was not capable of infecting human dendritic cells, the skilled worker could have readily modified the mutant according to known techniques. Undue experimentation would not be involved in determining which embodiments were inoperable.

15. In view of the foregoing facts regarding the routine nature of experimentation required to make and use the claimed recombinant alphaviruses, the extensive direction provided by the specification, the straightforward nature of the claimed subject matter, the high level of the skilled worker, the sophistication of the art, and the predictability of the art, it is my unequivocal opinion that the specification enabled, in April 1999, a skilled worker to make and use the compositions as claimed.

16. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

7/22/03
Date



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Professional Experience

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Principal Scientist, 1997 - 1999
Research Scientist II, 1995 - 1997

Viagene, Inc., Viral Therapeutics, San Diego, CA
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University of Southern California, School of Medicine, Los Angeles, CA
Postdoctoral Research Fellow, 1990 - 1994, Preceptor: Dr. Michael Lai

University of North Carolina, School of Medicine, Chapel Hill, NC
Graduate Research Assistant, 1988 - 1990, Preceptor: Dr. Robert Johnston

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Awards and Professional Associations

Registered Patent Agent, U.S. Patent and Trademark Office, #48738
NIH HIV Vaccine Design and Development Contract (6/00-5/05), co-PI
NIH AIDS Vaccine IPCAVD Grant (9/02-8/07), co-Investigator
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Education

Bachelor of Science, Microbiology - Auburn University, 1984
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Publications

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2. Gidwitz, S., **J.M. Polo**, N.L. Davis, and R.E. Johnston. 1988. Differences in virion stability among Sindbis virus pathogenesis mutants. *Virus Res.* 10:225-240.

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4. Johnston, R.E., N.L. Davis, **J.M. Polo**, D.L. Russell, D.F. Pence, W.J. Meyer, D.C. Flynn, L. Willis, S.-C. Lin, and J.F. Smith. 1990. Studies of alphavirus virulence using full-length clones of Sindbis and Venezuelan equine encephalitis viruses. pp. 334-339. In M. A. Brinton and F. X. Heinz (eds.), New Aspects of Positive Strand RNA Viruses. ASM Press, Washington, D.C.

5. **Polo, J.M.**, and R.E. Johnston. 1990. Attenuating mutations in glycoproteins E1 and E2 of Sindbis virus produce a highly attenuated strain when combined in vitro. *J. Virol.* 64:4438-4444.

6. Presley, J.F., **J.M. Polo**, R.E. Johnston, and D.T. Brown. 1991. Proteolytic processing of the Sindbis virus membrane protein precursor pE2 is nonessential for growth in vertebrate but is required for efficient growth in invertebrate cells. *J. Virol.* 65:1905-1909.

7. **Polo, J.M.**, and R.E. Johnston. 1991. Mutational analysis of a virulence locus in the E2 glycoprotein gene of Sindbis virus. *J. Virol.* 65:6358-6361.

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9. Stohlman, S.A., S. Kyuwa, **J. M. Polo**, D. Brady, M. M. C. Lai, and C. Bergmann. 1993. Characterization of mouse hepatitis virus-specific cytotoxic T cells derived from the central nervous system of mice infected with the JHM strain. *J. Virol.* 67:7050-7059.

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16. **Polo, J.M.**, and T. W. Dubensky, Jr. 1998. DNA vaccines with a kick. *Nature Biotech.* 16:517-518.

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18. Dubensky, Jr., T.W., **J.M. Polo**, and M. Liu. 1998. Live virus vaccines: something old, something new, something borrowed. *Nature Med.* 4:1357-8

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Patents / Applications

1. U.S. Patent 5,789,245 - Alphavirus structural protein expression cassettes.

2. U.S. Patent 5,814,482 - Eukaryotic layered vector initiation systems.

3. U.S. Patent 5,843,723 - Alphavirus vector constructs.

4. U.S. Patent 6,015,694 - Method for stimulating an immune response utilizing recombinant alphavirus particles.

5. U.S. Patent 6,015,686 - Eukaryotic layered vector initiation systems.

6. U.S. Patent 6,242,259 - Compositions and methods for packaging of alphavirus vectors.

7. U.S. Patent 6,329,201 - Compositions and methods for packaging of alphavirus vectors.

8. U.S. Patent 6,342,372 - Eukaryotic layered vector initiation systems for production of recombinant proteins.

9. U.S. Patent 6,376,236 - Recombinant alphavirus particles.

10. U.S. Patent 6,391,632 - Recombinant alphavirus-based vectors with reduced inhibition of cellular macromolecular synthesis.

11. U.S. Patent 6,426,196 - Alphavirus structural protein expression cassettes.

12. U.S. Patent 6,451,592 - Recombinant alphavirus-based vectors with reduced inhibition of cellular macromolecular synthesis.

13. U.S. Patent 6,458,560 - Recombinant alphavirus-based vectors with reduced inhibition of cellular macromolecular synthesis.

14. WO 96/21416, Methods and compositions for treatment of solid tumors in vivo, *Pending.*

15. WO 00/61770, Enhancement of the immune response for vaccine and gene therapy applications, *Pending.*

16. WO 00/61772, Compositions and methods for generating an immune response utilizing alphavirus-based vector systems, *Pending.*

17. WO 01/81553, Alphavirus-based vectors for persistent infection, *Pending.*

18. WO 01/92552, Methods for the purification of alphavirus replicon particles, *Pending.*

19. WO 02/26209, Microparticles for delivery of heterologous nucleic acids, *Pending.*

20. WO 02/80982, Nucleic acid mucosal immunization, *Pending.*

21. WO 02/99035, Alphavirus replicon particle chimeras, *Pending.*